ATS LABS

AMENDMENT TO GLP TEST PROTOCOL

Amendment No.:	1
Effective Date:	January 26, 2009
Sponsor:	Summit Brands 7201 Engle Road Fort Wayne, IN 46804-2228
Sponsor Representative:	Wagner Regulatory Associates P.O. Box 640 Hockessin, DE 19707-0640
Test Facility:	ATS Labs 1285 Corporate Center Drive, Suite 110 Eagan, MN 55121
Protocol Title:	Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Stainless Steel Surfaces
ATS Labs Protocol Number:	WRA01120908.NFS
ATS Labs Project Number:	A07219

Modifications to Protocol:

Due to the excessive foaming of the test substance during preparation, this protocol is amended to change second sentence of the product preparation on page 8 of the protocol from mixing the solution in a large flask using sterile bar for 3-4 minutes as follows:

Mix the solution in a sterile vessel by hand for 3-4 minutes.

Changes to the protocol are acceptable as noted.

Study Director _____ Date 1-26-09

EXACT COPY
INITIALS _____ DATE 2-10-09

(For Laboratory Use Only)
ATS Labs Project # A 07219
8/1/12/09

ATS LABS

PROTOCOL

Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Stainless Steel Surfaces

Test Organisms:

Staphylococcus aureus (ATCC 6538)
Klebsiella pneumoniae (ATCC 4352)

PROTOCOL NUMBER

WRA01120908.NFS

PREPARED FOR

Summit Brands
7201 Engle Road
Fort Wayne, IN 46804-2228

SPONSOR REPRESENTATIVE

Wagner Regulatory Associates
P.O. Box 640
Hockessin, DE 19707-0640

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PREPARED BY

Jill Ruhme, B.S.
Research Scientist I

DATE

December 9, 2008

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Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Stainless Steel Surfaces

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Fort Wayne, IN 46804-2228

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P.O. Box 640
Hockessin, DE 19707-0640

TEST FACILITY: ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PURPOSE
The purpose of this assay is to evaluate the antimicrobial efficacy of sanitizers on pre-cleaned or lightly soiled inanimate, nonporous, non-food contact stainless steel surfaces.

TEST SUBSTANCE CHARACTERIZATION
Test substance characterization as to content, stability, etc., (40 CFR, Part 160, Subpart F [160.105]) is the responsibility of the Sponsor. The test substance shall be characterized by the Sponsor prior to the experimental start date of this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to ATS Labs.

SCHEDULING AND DISCLAIMER OF WARRANTY
Experimental start dates are generally scheduled on a first-come/first-serve basis once ATS Labs receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is December 29, 2008. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of January 20, 2009. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

If a test must be repeated, or a portion of it, due to failure by ATS Labs to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test.

Neither the name of ATS Labs nor any of its employees are to be used in advertising or other promotion without written consent from ATS Labs.

The Sponsor is responsible for any rejection of the final report by the United States FDA or EPA concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the ATS Labs final report and notify ATS Labs of any perceived deficiencies in these areas before submission of the report to the regulatory agency. ATS Labs will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM
The U.S. Environmental Protection Agency requires that a specific bacterial claim for a sanitizer be supported by appropriate scientific data demonstrating the efficacy of the sanitizer against the claimed bacteria. This is accomplished in the laboratory by treating the target bacteria with the sanitizer (test substance) under conditions which simulate as closely as possible, the actual conditions under which the test substance is designed to be used. For sanitizer products intended for use on non-food contact surfaces, a carrier method is used in the generation of the supporting data. The test system to be used in this study will be a modification of the ASTM approved method for the evaluation of the antimicrobial efficacy of sanitizers on inanimate, nonporous, non-food contact surfaces.

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TEST PRINCIPLE

A film of bacterial cells dried on a surface of stainless steel carriers is exposed to the test substance for a specified contact time. After exposure, the carriers are neutralized and assayed for survivors. Appropriate, sterility, purity, carrier quantitation and neutralization controls are performed. The current version of Standard Operating Procedure CGT-4150 reflects the methods which shall be used in this study.

TEST METHOD

Test Organisms	ATCC #	Growth Medium	Incubation Parameters
Staphylococcus aureus	6538	Nutrient Broth	35-37°C, aerobic
Klebsiella pneumoniae	4352	Nutrient Broth	35-37°C, aerobic

Each test organism used in testing was obtained from the American Type Culture Collection (ATCC), Manassas, VA.

Carriers

1" x 1" stainless steel surfaces will be prepared by removing the adhesive protective backing, if applicable. Clean each carrier by dipping in ethyl alcohol and rinsing thoroughly in deionized water. After cleaning, decontaminate the surfaces by autoclave sterilization. (Alternatively, the carriers may be decontaminated by dipping in absolute ethanol and aseptically allowing the carriers to dry in a bio-safety hood.) Transfer the carriers aseptically to sterile Petri dishes.

Constant Humidity Chamber (Desiccator)

At least 1 day prior to use, fill the lower portion of a large size desiccator with about 500 mL of glycerin solution (approximately 86.5% glycerin in deionized water). This will provide a constant 40 ± 2% relative humidity at 35-37°C in which the inoculated glass carrier will be dried prior to treatment with the sanitizer. Replace the floor plate of the desiccator and store at 35-37°C to allow for equilibration. A controlled humidified chamber may be used in place of the desiccator.

Preparation of Inocula

K. pneumoniae and *S. aureus* – From stock cultures, inoculate 10 mL tubes of Nutrient broth. Incubate for 24 ± 2 hours at 35-37°C. Using a 4-mm inside diameter transfer loop, make at least three consecutive daily transfers of cultures in Nutrient broth prior to use as an inoculum. Transfer two loopfuls of culture to 10 mL of broth medium. Use transfer numbers 4-16 in testing. Use 48 ± 4 hour cultures for the inocula for this test.

Thoroughly mix the test organism culture on a "vortex" mixer, then allow the culture to settle for ≥15 minutes. Aspirate the upper two thirds of this suspension and use this as the inoculum for testing. An organic soil load may be added to the test culture per Sponsor's request.

Preparation of Test Substance

The test substance will be prepared according to the directions for intended use of the product. The test substance shall be used within three hours of preparation if additional preparation is required by ATS Labs.

Inoculation of Test and Control Carriers

Inoculate each sterile stainless steel carrier with 0.01-0.03 mL (10-30 µL) of culture using a calibrated pipettor. Spread inoculum to within approximately 1/8 inch of the edges of the square. Dry the inoculated carrier at 35-37°C in the constant humidity chamber or desiccator at 40±2% relative humidity with the lids slightly ajar. The lids may be left intact during drying if die-off is a concern. Allow the carriers to dry at this temperature and humidity for 20-40 minutes.

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Treatment of Inoculated Test Carriers
Following the completion of drying, remove all the plates from the drying chamber and transfer each carrier to a sterile vessel (i.e. jar).

Medicate each carrier using 5.0 mL of the test substance. Expose the carrier to the test substance at the desired temperature for the Sponsor specified exposure period.

Neutralization and Subculture
Following the Sponsor specified exposure period, transfer 20 mL of the appropriate neutralizer solution to the vessel. Rotate the vessel vigorously on an even plane approximately 50 rotations to suspend the surviving organisms in the neutralizer solution. Continue neutralizing each carrier, using identical staggered intervals. Within 30 minutes after neutralizing the carriers, plate 1.0 mL of the 10^0 and 10^{-1} dilutions in duplicate using the standard spread plate technique for all organisms.

Incubation and Observation
Incubate the *K. pneumoniae* and *S. aureus* plates at 35-37°C for 48±4 hours prior to observation for number of colonies. Subculture plates can be stored for up to 3 days at 2-8°C prior to examination. The plates will be visually enumerated following incubation or storage. Representative plates showing growth may be subcultured, stained and/or biochemically assayed to confirm or rule out the presence of the test organism. If possible, subcultures containing 30-300 colonies will be used for calculations.

TEST CONTROLS

Carrier Quantitation Control
Perform the test using inoculated carriers and sterile deionized water in place of test solutions. The inoculated control carriers will be exposed to the sterile deionized water for the identical time and temperature parameters used in the test procedure. Neutralize sterile deionized water as in the test. Prepare ten-fold serial dilutions of the neutralized broth. Plate appropriate dilutions in duplicate to yield countable numbers. Incubate plates as in the test and enumerate. If multiple time points are performed in testing, this control will be performed for the longest time point. The acceptance criterion for this study control is a minimum geometric mean of 2.51×10^4 CFU/carrier.

Purity Control
A "streak plate for isolation" will be performed on the organism culture(s) and following incubation examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Organic Soil Load Sterility Control
A one (1.0) mL aliquot of the soil used will be added to a tube of Fluid thioglycollate medium, incubated, and visually examined. The acceptance criterion for this study control is no growth.

Neutralizing Subculture Medium Sterility Control
A representative sample of uninoculated neutralizing subculture medium will be incubated and visually examined. The acceptance criterion for this study control is lack of growth.

Neutralization Confirmation
A neutralization confirmation control will be performed to demonstrate the neutralizer's ability to inactivate the test substance. The neutralization of the test substance will be confirmed by exposing sterile carriers to the test substance and neutralizing as in the test procedure. Transfer a 1.0 mL aliquot of a diluted suspension of the test organism to target approximately 100 CFU/mL of neutralized solution to the vessel and mix. Plate 1.0 mL of this mixed solution in duplicate. Perform a numbers control utilizing a sterile solution in place of the test substance. Incubate the resulting plates as in the test and enumerate. Note: If swarming is a concern, 0.1 mL aliquots may be plated. In this case, approximately 1000 CFU/mL will be targeted when adding organism to the neutralized solution.
NOTE: If multiple concentrations and/or exposure conditions are to be run in testing, only the shortest time point and/or the highest concentration needs to be evaluated in this control.

The acceptance criterion for this study control is growth within 1 $\log_{10}$ of the numbers control.

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Inoculum Count
Prepare and plate serial dilutions of the culture used as the inoculum using standard microbiological technique. Incubate the resulting plates as in the test, and then count the colonies to determine the number of organisms per milliliter of inoculum present at the start of the test. This control is for informational purposes only and therefore has no acceptance criterion.

Carrier Sterility Control
A representative uninoculated carrier will be added to the neutralizing subculture medium. The subculture medium containing the carrier will be incubated and examined. The acceptance criterion for this study control is lack of growth.

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM
ATS Labs maintains Standard Operating Procedures (SOPs) relative to efficacy testing studies. Efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including bacterial strains for purposes of identification, receipt and use of chemical reagents. These procedures are designed to document each step of efficacy testing studies. Appropriate references to medium batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each efficacy test is assigned a unique Project Number when the protocol for the study is initiated by the Study Director. This number is used for identification of the test subcultures, etc. during the course of the test. Test subcultures are also labeled with reference to the test organism, experimental start date, and test product. Microscopic and macroscopic evaluations of positive subcultures are performed in order to confirm the identity of the test organism. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: N/A

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
The test substance must meet the EPA efficacy data requirements that a 99.9% reduction in numbers of the test organism(s) was obtained as compared to the carrier quantitation control.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section.

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FINAL REPORT
The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the bacterial strains used, description of media and reagents, description of the methods employed, tabulated results and conclusion as it relates to the purpose of the test, and all other items required by 40 CFR Part 160.185.

PROTOCOL CHANGES
If it becomes necessary to make changes in the approved protocol, the revision and reasons for change will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

Standard operating procedures used in this study will be the current effective revision at the time of the work. Any minor changes to SOPs (for this study) or methods used will be documented in the raw data and approved by the Study Director.

PRODUCT DISPOSITION
It is the responsibility of the Sponsor to retain a sample of the test substance(s). All unused test substance will be discarded following study completion unless otherwise requested by Sponsor.

RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at ATS Labs. These original data include, but are not limited to the following:

1. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of final study report.
7. Study specific SOP deviations made during the study.

Facility Specific Documents

The following records shall also be archived at ATS Labs. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study specific SOP deviations made during the course of this study which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

REFERENCES

1. U.S. Environmental Protection Agency, Registration Division, Office of Pesticide Programs, Efficacy Data Requirements Sanitizer Test (for inanimate, non-food contact surfaces), DIS/TSS-10, January 7, 1982.

2. U.S. Environmental Protection Agency Pesticide Assessment Guidelines, Subdivision G, Section 91-2; Item j Sanitizers (for non-food contact surfaces) and Section 91-30(d) (8) Recommended Methods for Sanitizers – Non-food Contact Surfaces.

3. ASTM Test Method, Standard Test Method for Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces, E1153, July 2003.

4. Official Methods of Analysis of the AOAC, Seventeenth Edition, 2000. Chapter 6 – Disinfectants, 961.02. Germicidal Spray Products as Disinfectants.

DATA ANALYSIS

Calculations

Number of Organisms Surviving per Carrier

$$\text{CFU/carrier} = \frac{\text{(average number colonies/plate @ dilution) x (dilution factor) x (volume neutralized solution in mL)}}{\text{(volume plated in mL)}}$$

The carrier population will be calculated and reported using data from the most appropriate dilution(s).

Geometric Mean of Number of Organisms Surviving on Control Carrier:

$$\text{Geometric Mean} = \text{Antilog of } \frac{\text{Log}_{10}X_1 + \text{Log}_{10}X_2 + \text{Log}_{10}X_3}{3^*}$$

where X equals CFU/control carrier

Geometric Mean of Number of Organisms Surviving on Test Carrier:

$$\text{Geometric Mean} = \text{Antilog of } \frac{\text{Log}_{10}Y_1 + \text{Log}_{10}Y_2 + \text{Log}_{10}Y_3 + \text{Log}_{10}Y_4 + \text{Log}_{10}Y_5}{5^*}$$

where Y equals CFU/test carrier

**This value (or number of values for X and Y) may be adjusted to include the entire number of carriers evaluated in a set.*

Percent Reduction

% reduction = [(a - b) / a] x 100

where:

a = geometric mean of the number of organisms surviving on the inoculated control carriers.

b = geometric mean of the number of organisms surviving on the test carriers.

Recovery Log_{10} Difference = (Log_{10} Numbers Control) – (Log_{10} Neutralization Results)
Used for the neutralization confirmation control

Statistical Methods
Geometric Mean and Percent Reduction

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STUDY INFORMATION

(All sections must be completed prior to submitting protocol)

Sponsor (Date/Initial): 1/6/09 JMM

Test Substance (Name & Batch Numbers, *including ≥60 day old batch* - exactly as it should appear on final report):
Summit Brands Disposer Care Garbage Disposer Cleaner Sanitizer Disinfectant, Batch 08

Specify ≥60 day old batch: NA* *per email sent 1-6-09 JMM 1-8-09

Expiration Date: NA*

Product Description:

- ☐ Quaternary ammonia
- ☐ Iodophor
- ☐ Sodium hypochlorite
- ☐ Peracetic acid
- ☐ Peroxide
- ☑ Other sodium dichloro s-triazinetrione dihydrate

Test Substance Active Concentration (upon submission to ATS Labs): 2.0%

Neutralization/Subculture Broth:

- ☐ _____
- ☑ ATS Labs' Discretion. By checking, the Sponsor authorizes ATS Labs, at their discretion, to perform neutralization confirmation assays at the Sponsor's expense prior to testing to determine the most appropriate neutralizer. (See Fee Schedule).

Storage Conditions:

- ☑ Room Temperature
- ☐ 2-8°C
- ☐ Other: _____

Hazards:

- ☑ None known: Use Standard Precautions
- ☐ Material Safety Data Sheet, Attached for each product
- ☐ As Follows: _____

Product Preparation

- ☐ No dilution required, Use as received (RTU)
- ☑ *Dilutions/Concentrations to be tested see instructions below
  - ☐ Deionized Water (Filter Sterilized)
  - ☐ Tap Water (Filter Sterilized)
  - ☑ AOAC Synthetic Hard Water: 400 PPM
  - ☐ Other _____

Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.

Test Organism(s):
☑ *Staphylococcus aureus* (ATCC 6538)
☑ *Klebsiella pneumoniae* (ATCC 4352)

Carrier Number: 5 test carriers and 3 control carriers

Exposure Time: 30 seconds

Exposure Temperature: Room temperature

Organic Soil Load:

- ☑ Minimum 5% Organic Soil Load (Fetal Bovine Serum)
- ☐ No Organic Soil Load Required
- ☐ Other: _____

USE DILUTION INSTRUCTIONS - Dissolve the contents of one packet in 16 fluid ounces of AOAC Synthetic hard water heated to 48-51°C. Mix the solution in a large flask using a sterile bar for 3-4 minutes. Cut up the packet into ½ by ½ inch squares and add to the flask. Continue to stir briefly to thoroughly incorporate the the packet and any remaining powder. Allow the foaming to subside for 3-4 minutes. Prepared product should be made immediately prior to medication of test carriers. Entire preparation of the test substance should take approximately 10 minutes.

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TEST SUBSTANCE SHIPMENT STATUS

- ☐ Has been used in one or more previous studies at ATS Labs .
- ☑ Has been shipped to ATS Labs (but has not been used in a previous study).
  Date shipped to ATS Labs:_____ Sent via *overnight* delivery? ☐ Yes ☐ No
- ☐ Will be shipped to ATS Labs.
  Date of expected receipt at ATS Labs:_____
- ☐ Sender (if other than Sponsor):_____

COMPLIANCE

Study to be performed under EPA Good Laboratory Practice regulations (40 CFR Part 160) and in accordance to standard operating procedures.
☑ Yes
☐ No (Non-GLP Study)

PROTOCOL MODIFICATIONS

☑ Approved without modification per email sent 1-6-09 JM 1-8-09
☐ Approved with modification - Supplemental Information Form Attached - ☐ Yes ☐ No

APPROVAL SIGNATURES

SPONSOR:

NAME: Jim Wagner (Summit Brands) TITLE: Agent for Summit Brands

SIGNATURE: James Wagner DATE: 1/6/2009

PHONE: 302-234-8550 FAX: 302-234-7570 EMAIL: james@wagnerreg.com

For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.

Other individuals authorized to receive information regarding this study: ☐ See Attached

ATS Labs:

NAME: Jill Reckmeyer
Study Director

SIGNATURE: Jill Reckmeyer DATE: 1-8-09
Study Director

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